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Interleukin-10 Is a Mesangial Cell Growth Factor In Vitro and In Vivo

Steven James Chadban, Greg Hans Tesch, Rita Foti, Robert Charles Atkins, and David John Nikolic-Paterson

Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia

Macrophages are involved in the pathogenesis of mesangioproliferative glomerulonephritis. As macrophages are known to produce interleukin-10 (IL-10), we investigated the effect of recombinant murine IL-10 (rIL-10) on mesangial cell growth. In vitro studies were performed using the rat 1097 mesangial cell line. These cells exhibited a dose-dependent proliferative response to rIL-10 (23 % to 70 % at 80 ng/mL; $p < 0.01$), as assessed by both 3H thymidine uptake and cell count. This effect was inhibited by preincubation of rIL-10 with a neutralizing anti-IL-10 antibody. When added to cultures of growth-arrested 1097 cells, IL-10 induced dose dependent proliferation that paralleled the effects of platelet-derived growth factor. Incubation with a neutralizing anti-IL-10 Ab for 48 hours reduced 3H-thymidine uptake (median, 27% ; range, 2% to 56%) versus a control Ab; $p < 0.05$). Rat mesangial cells were also shown to express IL-10 mRNA and protein, as determined by Northern blotting and immunostaining, thereby suggesting a role for IL-10 in autocrine mesangial cell growth. To examine the effects of IL-10 in vivo, inbred male Sprague-Dawley rats were given subcutaneous rIL-10 (0.5 mg/kg) for 3 (n= 6), 7 (n= 3), or 14 days (n= 4), or vehicle control, then killed. IL-10 administration induced a transient reduction in creatinine clearance of 35% at Day 3 ($p < 0.01$). Following IL-10 administration, an increase in glomerular cellularity was seen, which was maximal at Day 3 (82.7 ± 5.9 nuclei/glomerular cross section versus control 64.6 ± 4.6 , 28% ; $p < 0.001$) and maintained at Day 14 (23 % ; $p < 0.01$). Immuno histochemical staining for proliferating cell nuclear antigen demonstrated an increased number of proliferating cells per glomerular cross section at day 3 (48 % versus controls; $p < 0.05$). Staining for alpha-smooth-muscle actin showed significant labeling only in the glomeruli of IL-10-treated animals; double-labeling with an anti- proliferating cell nuclear antigen Ab demonstrated that some of these mesangial cells were proliferating. Collectively, these results suggest that IL-10 is a growth factor for rat mesangial cells both in vitro and in vivo. (Lab Invest 1997, 76:619-627)

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Direct protective effect of interleukin 10 on articular chondrocytes in vitro

WANG Yueqing 王跃庆 and LOU Siqun 娄思权

Department of Orthopaedic Surgery, Third Hospital, Peking University,
Beijing 100083, China (Wang YQ and Lou SQ)

Keywords: interleukin 10 • chondrocytes • cell culture •
nitric oxide synthase • matrix metalloproteinase

Objective To assess whether interleukin 10 (IL 10) is chondroprotective in vitro

Methods Chondrocytes were isolated from femoral cartilage of rats (7-10 days) by digestion with collagenase II. The first passage cells were grown in 24 well plates with DMEM, supplemented with 10% fetal bovine serum, for 2-4 days. The cells were then cultured in 0-1% fetal bovine serum DMEM medium, and given respectively interleukin 1 (IL 1) 100 µ/ml, IL 1 100 µ/ml+recombinant murine interleukin 10 (rmIL 10) 20 ng/ml, rmIL 10 20 ng/ml, and cultured for 48 hours. Scanning electron morphology and immunohistochemical study of nitric oxide synthase 2 and matrix metalloproteinase 3 mRNA in situ hybridization were performed. Cell proliferation and morphology were observed under inverted microscope from the beginning of cell culture for three weeks.

Results IL 1 stimulated granule production in the cytoplasm of chondrocytes, and the cells died in the second and third weeks of culture. IL 10 antagonized IL 1, protected the cells from death and maintained chondrocyte proliferation. Scanning electron morphology showed that IL 1 stimulated the formation of numerous microvilli on the cell surface, while thin and less numerous microvilli were found in cultures with

IL 10 Immunohistochemical study and in situ hybridization showed that IL 10 inhibited NOS2 and MMP3 expression

Conclusion IL 10 not only inhibits the synthesis of inflammatory cytokines, but also directly protects chondrocytes by antagonizing IL 1

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Interleukin (IL) 10 is a potent anti-inflammatory cytokine. It inhibits the production of a wide range of cytokines such as IL 1, TNF α , and granulocyte macrophage colony stimulating factor (GM-CSF) [1,2]. It also has been shown to induce immune suppression via the inhibition of MHC class II expression, effecting anti-inflammation. IL 10's effects in these areas involve hematopoietic cells including monocytes/macrophages, polymorphonuclear leukocytes, and Th1 and Th2 cells. IL 10 has also been shown to influence the functions of nonhematopoietic cells [3-5]. Studies indicate that IL10 has potential therapeutic uses in the treatment of chronic inflammatory disorders such as rheumatoid arthritis and osteoarthritis [6,7]. Besides its effects on hematopoietic cells, whether IL 10 has any direct effects on chondrocytes is an important question in its future clinical use. The purpose of this study was to examine the direct effect of IL 10 on chondrocytes.

METHODS

Chondrocyte preparation

Chondrocytes were obtained from Wistar rats 7-10 days after birth. Cartilage from femoral condyles was collected, and chondrocytes were isolated by digestion with type II collagenase for 4 hours at 37°C. The cells were grown in tissue culture flasks containing DMEM medium, supplemented with 10% fetal bovine serum, and kept in a 37°C humidified incubator with 5% CO₂ for 7-10 days. The first passage cells were then transferred to 24-well plates with glass cover slips for further culturing in the same medium and environment for 2-4 days. The cells were incubated in DMEM with 0-1% fetal bovine serum, with separate groups receiving the following cytokines for further investigation: IL 1 100 μ /ml (Zhongshan Co. Ltd,

Beijing, China); IL-1 100 μ g/ml + rm IL-10 20 ng/ml (Peprotech, England); and rm IL-10 20 ng/ml

Morphological observation

Cell morphology was observed under an inverted microscope (Olympus) from the beginning of the cell culture for 3 weeks. After 48 hours of culturing with cytokines, scanning electron microscopy was performed.

Immunohistochemistry

Chondrocytes cultured for 48 hours with cytokines were fixed in 1:1 methanol:acetone and blocked with serum. Then, they were labeled with rabbit anti-rat nitric oxide synthase 2 (NOS2) for 30 minutes at 37°C and washed in BPS and endogenous peroxidase inactivated in 0.3% H₂O₂ in methanol. They were then incubated with biotin conjugated goat anti-rabbit IgG followed by streptavidin peroxidase complexes, and developed with DAB to produce a brown color.

In situ hybridization

Chondrocytes cultured for 48 hours with cytokines were fixed in 1:1 methanol:acetone, washed in BPS with DEPC, digested with proteinase K, and further fixed in paraformaldehyde. Then, they were washed in BPS with DEPC, hybridized with digoxigenin labeled probes [matrix metalloproteinase 3 (MMP3) 1.1 Kb, Peking University Medical Center, China] overnight at 48°C, and then incubated with alkaline phosphatase labeled anti-digoxigenin Fab segments (1:500) for 30 minutes at 37°C and developed with nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate indimethylformamide overnight at room temperature in a dark room.

RESULTS

Chondrocyte morphology

Inverted microscope view

Chondrocytes cultured with IL-1 showed many granules in their cytoplasm in the first week, while other cells took on clear appearances. In the second and third weeks, many cells died in the culture with IL-1. There was less or no cell death in

cultures with IL 1+IL 10 or IL 10 alone, and cell proliferation could still be seen in cultures with IL 1+IL 10

Scanning electron microscopy

Chondrocytes cultured for 48 hours with IL 1 showed numerous microvilli protruding from their cell surfaces. Short and thin microvilli, much less numerous, were seen in cultures with IL 1+IL 10 or IL 10 alone.

Immunohistochemistry

Strong expression of nitric oxide synthase appeared in cells cultured with IL 1. However, cells cultured with IL 1+IL 10 or IL 10 alone showed decreased expression and they were clear in appearance.

In situ hybridization

Strong expression of MMP3 appeared in cells cultured with IL 1. Cells cultured with IL 1+IL 10 or IL 10 alone showed decreased MMP3 expression.

DISCUSSION

IL 10 is a product of Th2 cells, monocytes and B cells. It has been shown to exert a number of immunoregulatory and anti-inflammatory effects. IL 10 inhibits the synthesis of IL 1, IL 6, IL 8, and TNF α by activated macrophages, and downregulates MCP 1 expression. Moreover, IL 10 inhibits matrix metalloproteinase 3 production and NOS2 expression.

All these factors have been demonstrated to induce cartilage destruction in rheumatoid arthritis and osteoarthritis.

IL 10 has been shown to be chondroprotective, and may have a potent effect antagonizing inflammatory cytokines in rheumatoid arthritis and osteoarthritis therapy in the future. Previous studies have shown that the effect of IL 10 on hematopoietic cells is indirect. Neutralizing endogenous IL 10 results in an increase of IL 1 and TNF α release by synovial cells. In the collagen-induced arthritis model, IL 10 with IL 4 reduces mononuclear cell infiltration and decreases cartilage destruction. It has been demonstrated that IL 10 decreases engrafted cartilage erosion by inhibiting mononuclear cell recruitment in vivo. These studies

indicated that IL-10 indirectly protects cartilage by regulating hematopoietic cells. Whether IL-10 has any direct effects on chondrocytes is unknown.

This paper has demonstrated that IL-10 has a direct chondroprotective effect. In our experiments, IL-10 antagonized IL-1 and protected chondrocytes from destruction and maintained cell proliferation. The expression of NOS2 and MMP3 was downregulated. The mechanism remains unclear, but one explanation might be that IL-10 stimulates the synthesis of IL-1 receptor antagonist (IL-1ra), induces the expression of the tissue inhibitor of metalloproteinase-1 (TIMP-1), and acts as a growth factor [4].

IL-1 stimulates nitric oxide synthase and MMP expression, which plays a key role in cartilage degradation in rheumatoid arthritis and osteoarthritis. This paper provides the first evidence that IL-10 directly antagonizes IL-1 and has a protective effect on chondrocytes, giving new information for future clinical application in rheumatoid arthritis and osteoarthritis therapy.

Different effects of IL-10 on different cells have been reported previously. IL-10 has been shown to slow the growth of T cells, but stimulate the growth of B cells and mesangial cells. IL-10 also regulates chemokine release by mononuclear cells and fibroblasts. It even regulates the same cell in its various stages [10]. In the present study, it was shown that IL-10, even though chondroprotective, induced some NOS2 and MMP3 expression, though not sufficient to cause cell damage.

This fact indicates that IL-10 may have dual or contradictory effects on the cell.

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